Developmental Cell *18*

Supplemental Information

Arabidopsis Regeneration from Multiple Tissues Occurs via a Root Development Pathway

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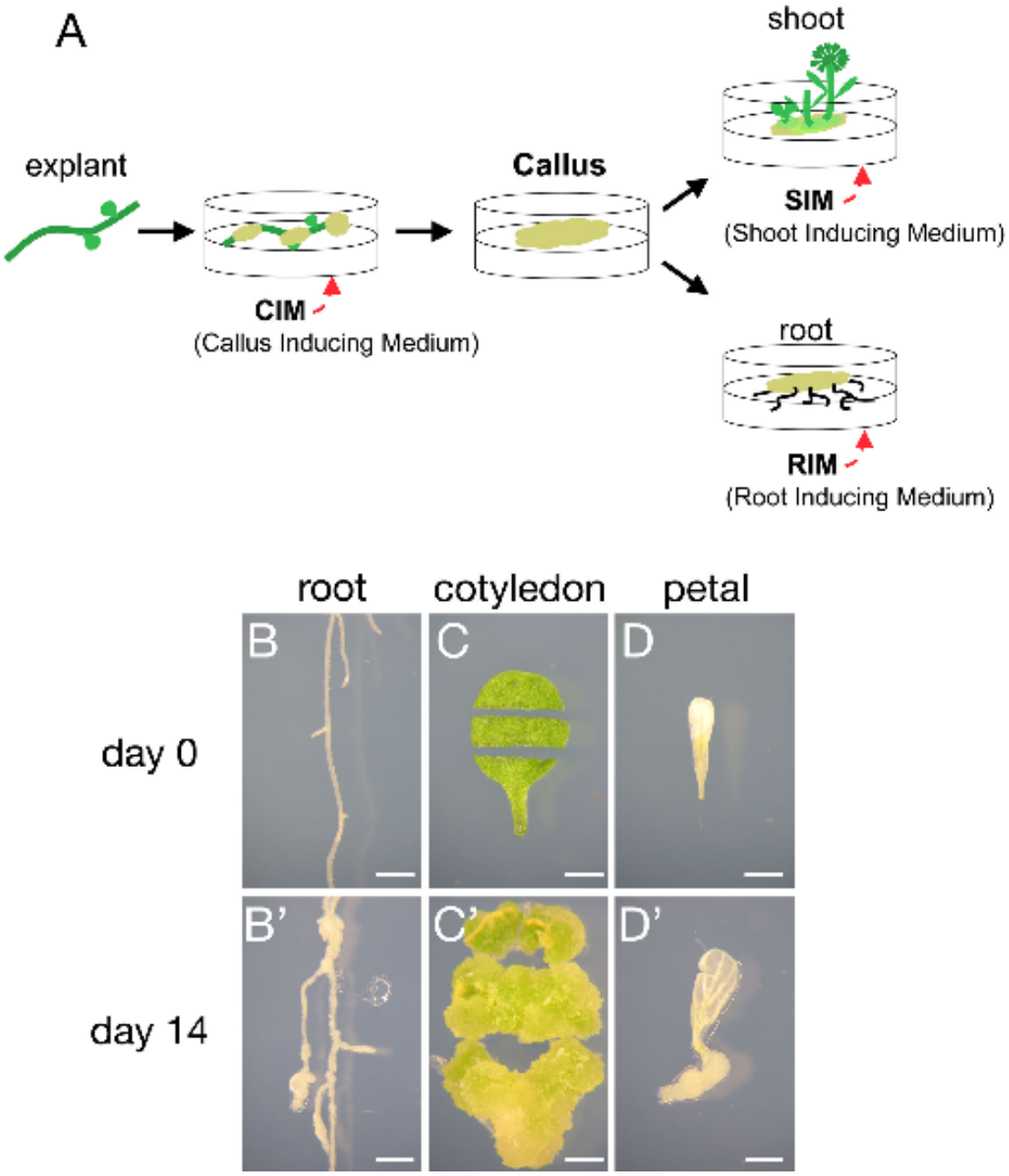

Figure S1. Schematic diagram of the plant regeneration assay and the callus derived from different organs, related to Figure 1

(A) Procedure of the plant regeneration assay. CIM, SIM, and RIM are abbreviations for Callus-, Shoot-, and Root inducing medium respectively. (B-D') Explants derived from different organs. Untreated explants (B-D) and explants treated with CIM for 14 days (B'-D') were derived from root (B, B'), cotyledon (C, C') and petal (D, D'). Scale bars: 1 mm.

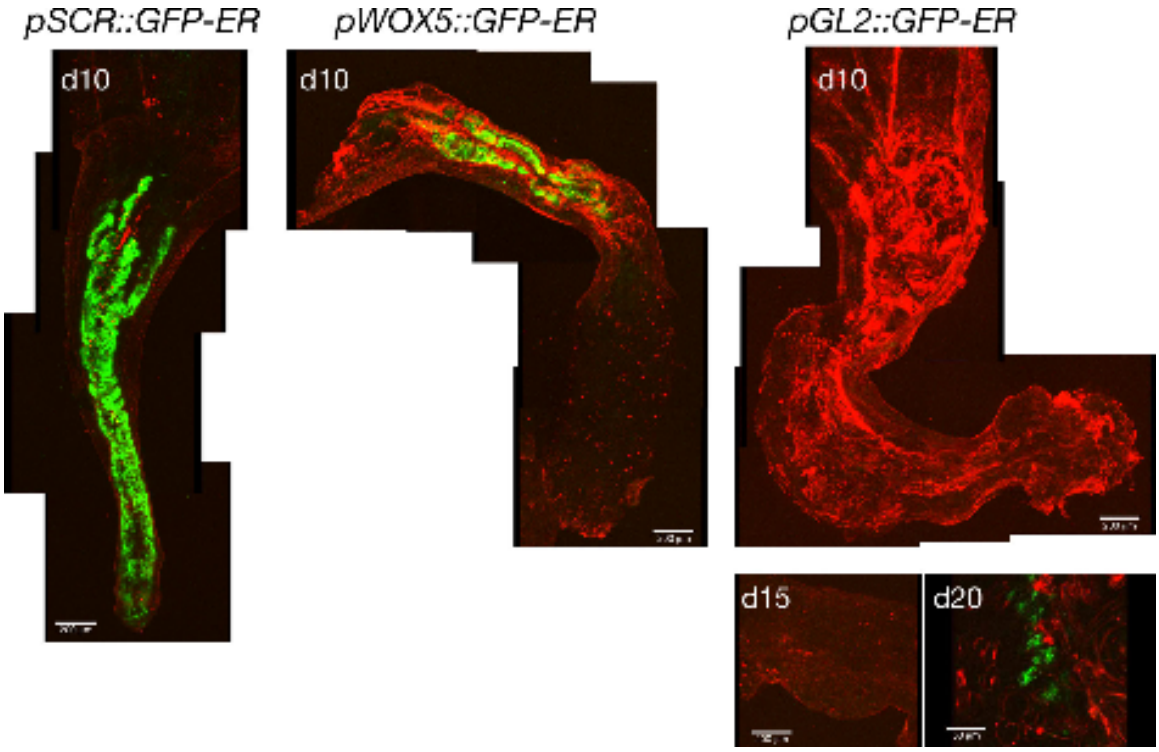

Figure S2. Callus derived from petal, related to Figure 3

Root tissue marker expression (green) in callus derived from petal. The number of days of CIM incubation is indicated at the top left corner of each panel. Cellular outlines were visualized with propidium iodide staining (red). Callus was formed mainly around the vein or stalk regions. The order of marker initiation was similar to that of root callus. *pGL2::GFP-ER* reporter started to be expressed between day 15 and day 20 on CIM, while *pSCR::GFP-ER* and *pWOX5::GFP-ER* expression was detected before day 5.

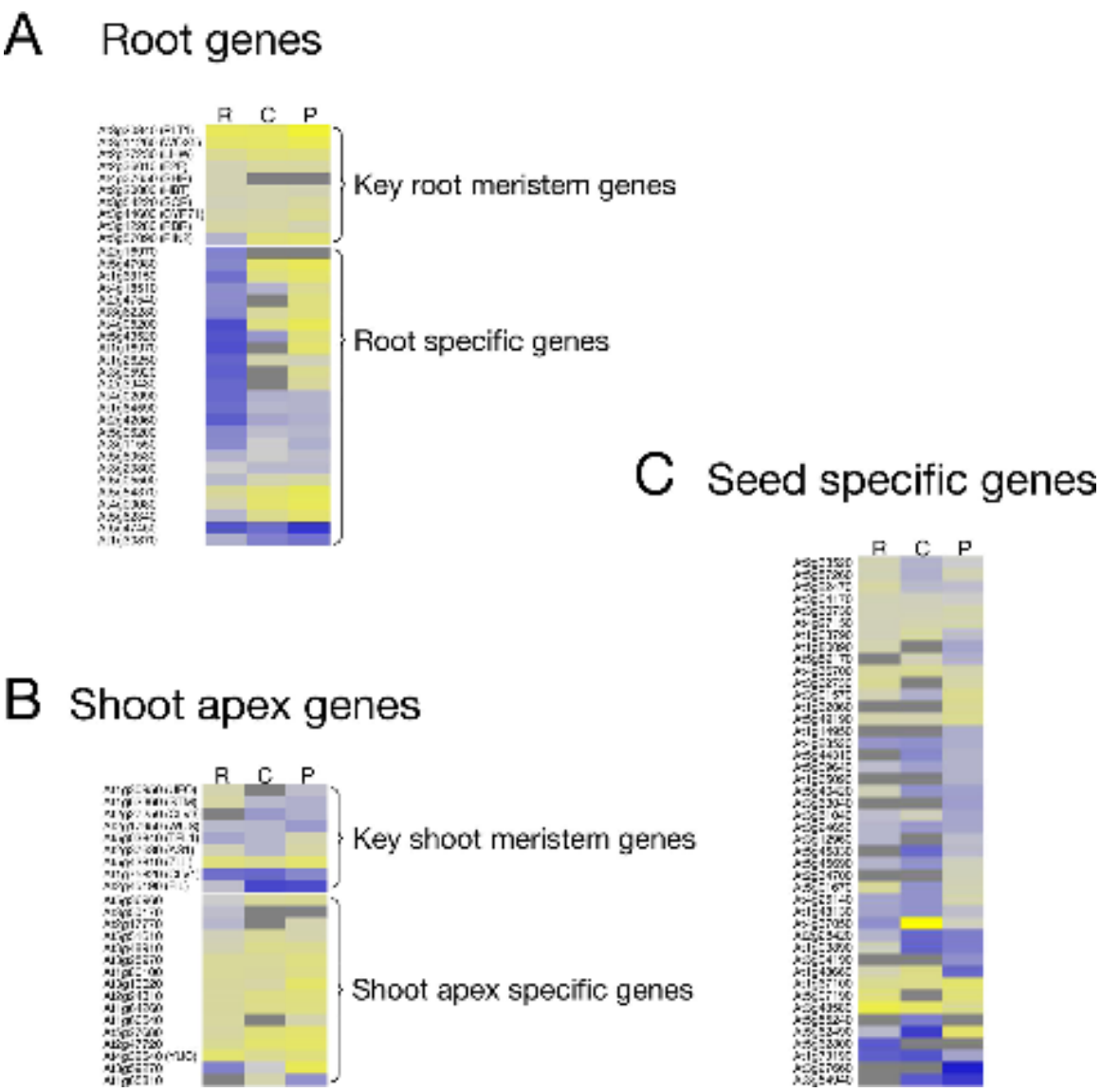

Figure S3. Expression ratios for the root, shoot apex and seed genes in explants, related to Figure 4

Clustering displays of expression ratios (callus versus its original tissue) for key genes for root meristem development and root specific genes (**A**), key genes for shoot meristem development and shoot apex specific genes (**B**) and seed specific genes (**C**) in each of the three experiments (R, C, P indicate root, cotyledon, petal explants respectively). The lists of root and shoot apex specific genes were from (Schmid et al., 2005) and seed specific genes were from (Becerra et al., 2006; Schmid et al., 2005).

Note that many of the root-specific genes are up-regulated during callus formation in cotyledon and petal explants but not in root explants.

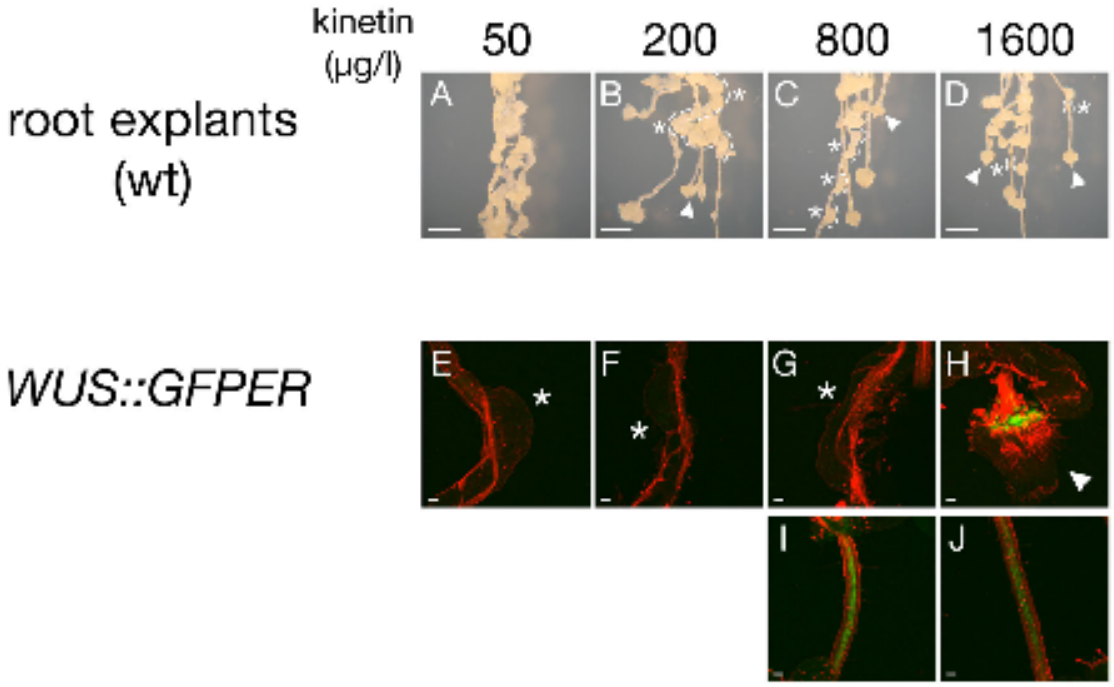

Figure S4. Novel type of callus is also induced in the high-kinetin media, related to Figure 6

Root explants of wild-type (A-D) and *pWUS::GFP-ER* (E-J) treated with CIM containing different concentration of kinetin, 50, 200, 800 and 1600 µg/l (columns are in this order from the left). All media contain the same concentration of 2,4-D: 500 µg/l. The explants in (A-D) were cultured for 11 days and in (E-H) for 10 days. In the standard CIM (A, E), a broad region derived from the body of the root grew and formed callus, while in CIM with a high concentration of kinetin (B-D, F-H), in addition to this same type of callus (asterisks), another type of cell mass was also induced, which was cone- or round-shaped and formed nearer the root tips (arrowheads). The *WOX5::GFP-ER* reporter was expressed in the former type of callus (Figure 6). The latter type of outgrowth expressed the shoot meristem marker *WUS::GFP-ER* instead of *WOX5* reporter (H). *WUS* reporter signal was also detected in non-growing trunk regions of root explants in the two high kinetin conditions (I, J). Scale bars in (A-D), 1 mm; (E-J), 50 µm.

Table S2. *alf4-1* suppresses callus formation in the three different organs, related to Figure 5

	***alf4-1* -/-**	***alf4-1* +/-**	**wild type**
root	3.5% (n = 29)	90.2% (n = 41)	96.8% (n = 31)
cotyledon	7.7% (n = 39)	87.8% (n = 33)	93.8% (n = 48)
petal	0% (n = 41)	93.8% (n = 48)	97.8% (n = 46)

(%) The ratio of the explants or leaves forming callus.

The number of root and petal explants forming callus was counted. In the case of cotyledon, three explants were made from a single leaf. The number of leaves, at least one of the explants of which formed callus, was counted.